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Western Palearctic water frogs (Amphibia, Ranidae): Systematics, genetics and population compositions

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Summary. Recent studies of the western Palearctic water frogs revealed the occurrence of at least 5 types of water frogs (*Rana ridibunda, R. lessonae, R. perezi,* 1 Italian and 1 Balkan species) as well as 4 types of their hybrids. In addition to the systematics, genetics and population compositions of these frogs, the complex mechanisms that lead to the production of the hybridogenetic hybrid *Rana esculenta* are emphasized, and the unsolved problems relating to the control of the hybridogenetic process are discussed.

In his book entitled, 'Die Rassenbildung bei europäischen Rana-Arten und die Gültigkeit der Klimaregeln', Kauri[2] proves that all western Palearctic water frogs belong to one species *Rana esculenta* Linnaeus. His conclusion ended the period of classic taxonomical study of these animals. The discovery in Poznań, Poland, of water frogs of 3 morphological types[3], of which 1 intermediate form, *R. esculenta* Linnaeus, is a hybrid of the 2 others - *R. ridibunda* Pallas and *R. lessonae* Camerano[4,5] - marked a turning-point for the future study of these frogs. This finding, which was confirmed by many authors[6], acted as a stimulus for intensive research, the results of which were presented in a Symposium in Berlin, 1978[7].

At present[8-13], we know that at least 5 species of water frogs and 4 types of their hybrids (table) inhabit Europe. Some additional species and their hybrids live in north-western Africa[14,15] and may be in other broad areas[10].

Results from past studies indicate that the species of western Palearctic water frogs form a group of animals whose hybrids are characterized by unusual genetic properties. It is impossible to discuss all their features in this short article so we shall concentrate on the most important information that has been revealed by severeal major investigations.

Systematics. The systematic status of the 3 taxons of frogs from the Poznan vicinity was established on the basis of the characteristics of hybrids of various generations[4,5,16-18]. These results were confirmed by electrophoresis of serum proteins[19,20]. Electrophoretic methods now play an essential role in further investigations because frogs of southern Europe, north-western Africa, and south-western Asia are similar to each other according to their external features and resemble *ridibunda.* Analysis of electrophoretic protein phenotypes of southern Europe and north-western Africa have led us to the following conclusions: 1. The Italian peninsula, Corsica and Sicily, are inhabited by mixed populations of the Italian species and its hybridogenetic hybrid[9]. Still other forms of frogs may live[10,21] in these territories. 2. Southern France and the north-eastern Iberian Peninsula are inhabited by *perezi* and its hybridogenetic hybrid[8,22]. 3. The Balkan Peninsula is inhabited by mixed populations of 3 taxons: *ridibunda,* Balkan species and their hybrid[12,13]. 4. Some forms and probably some types of their hybrids live in north-western Africa[13,14].

The systematic status of the Italian and Iberian taxons (2 species and their 2 hybridogenetic hybrids) was corroborated by crossing experiments[11]. Electrophoretic protein phenotypes of both hybrids are similar to those of *esculenta*[8,9]. This suggests that their second parental species, as in the *esculenta* hybrid, was also *ridibunda* (table) which now lives allopatrically. This conclusion needs confirmation, and establishment of the relationship of the forms living in the Balkan Peninsula and north-western Africa needs further study. Of some assistance in this type of research are studies of immunological distances between the serum albumins of different frog forms[15,23] which can help to estimate their relationship and also their times of divergence.

Genetic systems. In RL, RI and RP hybrids (table) the independent segregation of chromosomes was replaced by the hybridogenetic system[24-26], or a sys-

Five species of European water frogs and their hybrids living in nature. All taxons were bred in Poznań for some years already. Hybrids were derived as a rule by crossing *R. ridibunda* females with *non-ridibunda* males. The *ridibunda* × *lessonae* crosses form a series of diploid (RL) and triploid (RRL and RLL) hybrid lineages which are collectively known as *Rana esculenta*. Some other taxons (2 species and 3 hybrids) have no name as yet. F_1, first generation hybrids; B_n, different generations of backcross hybrids

Parental species Female	 Male	Hybrids Genomes received from parents and symbols of generations		Origin and persistance in nature by
R. ridibunda-RR ×	*R. lessonae*-LL	RL	F_1	Hybridization
		RL, RRL, RLL,	B_n	Hybridogenesis
R. ridibunda-RR ×	*R. perezi*-PP	RP	B_n	Hybridogenesis
R. ridibunda-RR ×	Italian species-II	RI	B_n	Hybridogenesis
R. ridibunda-RR ×	Balkan species-BB	RB	F_1	Hybridization

tem of genome segregation[27]. The genome of one parental species is eliminated from the germline premeiotically[28-32], whereas the non-recombined genome of the other species[25,27,33,34] which undergoes premeiotic haploidization with subsequent premeiotic rediploidization, and consequently normal meiosis is restored in the gametes. In RI and RP hybrids the process is simple. Only *ridibunda*-like genomes are transmitted clonally to the gametes. However, in the *esculenta* hybrid, which contains different series of individuals of various origin and ploidy, this process is complex. In mixed *lessonae*/*esculenta* populations (L-E system[27]) which inhabit all central Europe and adjacent countries[10, 35,36], the *ridibunda* genome is transmitted to gametes, and in mixed *ridibunda*/*esculenta* populations (R-E system) which are discovered in the Lower Oder River only[37,38] the *lessonae* genome is passed to gametes[39]. In both systems there are exceptions to which we will return later. In these populations the *esculenta* individuals are as a rule backcrosses with those species from which genomes were eliminated; they rarely originate as F_1 hybrid[36,40] (cf. table).

Populations. Water frog populations in central Europe show very high differentiation in the composition of phenotypes, ploidy and sex. In *lessonae*/*esculenta* populations the numbers of the individuals of both taxons are different as a rule. In some of the percentage proportions of *lessonae* to *esculenta* individuals are 95:5, and in others this is reversed. Our studies[41] of some ponds near Poznań show stability of the phenotype structures in such populations. Sex proportions in both taxons are equal (1:1). Populations near Neusiedlersee, Austria[26,33], are the only exception to the rule; the sex ratio in *esculenta* is extremely biased in favor of females[44]. These facts suggest[26] that *lessonae* males of these populations are homogametic frogs; crossing experiments with them also yielded very few males[29]. It is very difficult to explain why in the progeny from crosses *lessonae* males from Neusiedlersee and *esculenta* females from Poznań males predominate[43].

In *ridibunda*/*esculenta* populations only males appear[37] in *esculenta* phenotype; their gametes contain the *lessonae* genome with male chromosomes that assure the existence of the male line of the *esculenta* phenotype. There are also individuals which produce gametes with *ridibunda* and female chromosomes as well as those which produce both types of genomes simultaneously[39]. The mechanism of this phenomenon is not known and the observations described should be confirmed by far more data.

There are also pure *esculenta* populations in Meclemburgia, in the northern part of the German Democratic Republic[44], and in Skåne, southern Sweden[45] in which most individuals are triploid[27,44,46]. Reproduction in these populations has not been fully explained. According to Dr Günther[47] the triploid frogs produce gametes with *lessonae*, and the diploid ones produce 2 types of gametes: haploid with *lessonae*, and diploid with *ridibunda*/*lessonae* genomes. This shows that certain types of individuals adopt the function of parental species in these populations.

Presumably, explanation of the phenomena described should be sought in *esculenta* populations belonging to L-E system, since in the progeny of some crosses individuals with unexpected features appear[33,43]. The phenomena are caused by disturbances in gametogenesis leading to, among other features, strongly differentiated[48] egg sizes characterized by different ploidy and different genomes[49]. Together with eggs of *ridibunda* genome, which contain as a rule above 99% of all gametes, haploid and diploid gametes with L, LL, RL and RR genomes were also found[50,51]. Consequently, we can suppose that the same phenomena should exist in male gametes as well[52]. If this is true we can expect progeny with extremely differentiated characteristics.

The above observations were of special meaning because they were confirmed in nature in an isolated *esculenta* population in Turew near Poznań[51]. In 1977-1981 the population consisted of about 800 adults[53]. During 5 years of observations their phenotype and genotype structures were stable (about 95% RL diploids and 5% RRL triploids); however, the structure of metamorphosed individuals was highly differentiated. Every year the females laid hundreds of thousands of eggs from which only a small percent of individuals metamorphosed. They were *esculenta* and *ridibunda* phenotypes with RL, RRL and RR genomes (cf. table). In some years we observed also single triploid RLL and diploid LL froglets. In spite of such differentiation of the young animals the composition of the adults was similar (RL and RRL) during 5 years of observations.

We cannot yet fully explain this phenomenon either; nevertheless, based on these observations, we can draw the conclusion that in *esculenta* populations of L-E system, these genetic systems of different origins and the biotopes are an indication of their fitness in nature.

Conclusion. The understanding of the hybridogenesis phenomenon and, above all, the discovery of the factors which control this process are two of the most important problems that remain to be solved. We know at present that in gametogenesis of the *esculenta* individuals the *lessonae* genome or the *ridibunda* genome is eliminated from the germ line. We know also that this process is not always precise: the *esculenta* males of R-E system which produce 2 types of gametes illustrate this clearly. Data from these animals prove that both genomes undergo the whole process of meiosis, but we cannot, at present, explain how it proceeds. We may, however, believe that premeiotic separation of *lessonae* and *ridibunda* genomes (premeiotic haploidization) exists which is followed by the process of premeiotic rediploidization. The subsequent meiosis and formation of gametes with different genomes proceed independently. Fluorescence double staining techniques[32] which enable differentiation of all *ridibunda* chromosomes from all *lessonae* chromosomes can facilitate the solution of these problems.

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Cerebral decarboxylation of *meta*- and *para*-tyrosine

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Summary. The decarboxylase inhibitor DL-*α*-monofluoromethyldopa reduces, in a dose dependent manner, the concentration of striatal *p*-tyramine in the mouse. Homovanillic acid is also significantly reduced. Conversely, this treatment increases the *m*-tyramine concentration. Administration of *m*-tyrosine produces large increases in *m*-tyramine and a slight decrease in *p*-tyramine; these changes are potentiated in the presence of the decarboxylase inhibitor. Such data along with other recently published results permit the conclusion that *m*-tyramine arises from phenylalanine via *m*-tyrosine and that *p*-tyramine arises by decarboxylation of *p*-tyrosine. Both these reactions are closely related to the activity of tyrosine hydroxylase and the availability of appropriate substrates.

The formation of the putative neurotransmitters dopamine (DA) and 5-hydroxytryptamine (5-HT) is via decarboxylation of their respective hydroxylated precursor amino acids, DOPA and 5-hydroxytryptophan[4,7,78]. These particular amines are concentrated in quite significant amounts (i.e. μg/g quantities) in parts of the mammalian brain (basal ganglia, mesolimbic system, hypothalamus, etc.)[2,5]. The enzyme responsible for their formation, aromatic L-amino acid carboxylyase (EC 4.1.1.28) is fairly ubiquitously distributed, although there is some doubt as to whether it is a single protein[72,73].

We have been interested for some time in a related group of biogenic amines that have been dubbed erroneously the trace amines because of their tiny tissue concentrations (low ng/g)[9,11,20]. By employing fairly sophisticated mass spectrometric techniques it has proved possible to identify and quantitate several of these trace amines, *β*-phenylethylamine (PE)[35], tryptamine (T)[66] and *meta*- and *para*-tyramine (mTA and pTA)[65,67] and to demonstrate their heterogenous distribution throughout the brain[21,35,51,65-68] and within the cell[13,14].

There seems little doubt that PE and T are formed directly by decarboxylation of phenylalanine and tryptophan respectively[29,61]. The case for the TA's is more complex since *meta*-tyrosine has not hitherto been thought to be a normal tissue component, *para*-tyrosine decarboxylation appears to be very slow[43] and both mTA and pTA can be formed by hydroxylation of PE[15,18,30,38] and dehydroxylation of dopa and dopamine[15,25-27,38] both peripherally and centrally.

Despite their tiny concentrations, however, these biogenic trace amines are interesting because they exhibit very fast turnover rates[21,34,64,75,76], increase differentially and markedly their tissue and body fluid levels after monoamine oxidase blockade[64] and their deaminated catabolites, phenylacetic acid, indoleace-